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Communications

Peptoid-based Positional Scanning Derivatives: Revealing the Optimum Residue Required for Ice Recrystallization Inhibition Activity for Every Position in the AFGPs

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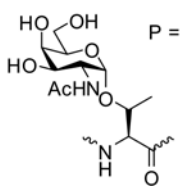
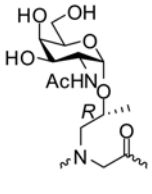
Antifreeze glycoproteins (AFGPs) bind to the ice crystals, thereby inhibit ice crystal growth. The discovery of AFGPs in the blood serum of fish by De Vries demonstrated that AFGPs is an essential biomaterial for fish to survive at sub-zero temperature in the Antarctic Sea.[1] This unique property of AFGPs has attracted significant interest due to their potential application in a variety of fields including medicine and the frozen-food industry. AFGPs were classified into 8 subclasses, in which AFGP1 has the largest (33.7 kDa), and AFGP8 has the lowest molecular weight fraction (2.6 kDa). Among AFGPs, AFGP8 is a good candidate as biomaterial due to the lowest molecular weight (2.6 kDa) that led to the intensive studies on the synthesis and activity of AFGP8-related compounds by several research groups,[2] to develop efficient and cost-effective mass production of AFGPs with high purity as well as to understand the mechanism of action of AFGP8. AFGP8 was consists of repeating tripeptide units, Alanyl-Alanyl-Threonyl (Ala-Ala-Thr)$_{n=4}$ units, connected with the disaccharide β-D-galactosyl-(1→3)-α-D-*N*-acetyl-galactosamine through a glycosidic bond at the hydroxyl group of the threonine residue. Although AFGP8 is a good candidate for the medical and industrial applications, one of the greatest huddles has been to achieve stability. As a part of our continuing efforts toward the rational design of AFGP mimics possessing enhanced stability,[3] we have developed a low-cost synthetic strategy to afford simple structural mimics of AFGP8. Our approach utilized the peptoids, or *N*-substituted oligoglycines in which sugar moiety is moved into the amide nitrogen atom from the α-carbon atom of each threonine residue. Peptoid bond is well known to have a more resistant proteolytic degradation and enhance the cell permeability compare to natural peptide bond. We prepared a series of AFGP8 analogues by incorporating glyco-peptoid monomer (**P**) and carried out the positional scanning in order to figure out the distinct position of peptoid mimics in the native AFGP with either increase or retain in activity. Toward this end, the inherent instability of the C-O glycosidic bond was recently overcome by rational design of carbon-linked AFGP analogues in which the shortest distance of two CH_2 groups between the sugar moiety and the peptide backbone gave the strongest RI activity.[4] Moreover, Norgren *et al.* describe the use of click chemistry to synthesize peptoid analogues of AFGPs, but none of them exhibited the anti-freeze activity.[5] This could be due either to the nature of the triazole act as a constraint or to the different conformations of the peptoid fragment. In a hope to eliminate bias of the direct evaluation of glycopeptoid AFGP mimics, here we attempted to synthesize and examine the effect of *N*-linked glycopeptoid on AFGP analogue having no constraints in

Table 1. Positional scanning using glycopeptoid momomer

AFGP	Sequence	M.W.
1	AAT AAT AAT AAT AA	1946
2	AA**P** AAT AAT AAT AA	1960
3	AAT AA**P** AAT AAT AA	1960
4	AAT AAT AA**P** AAT AA	1960
5	AAT AAT AAT AA**P** AA	1960
6	AAP AAP AAP AAP AA	2002

A = Ala

T = 

P = 

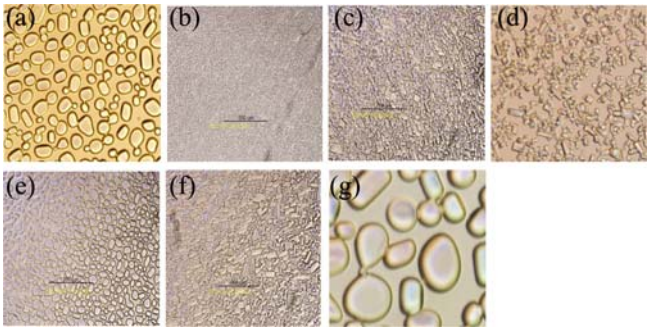


Figure 1. Ice recrystallization inhibition activity in the presence of (a) D_2O (b) AFGP1 (c) AFGP2 (d) AFGP3 (e) AFGP4 (f) AFGP5 (g) AFGP6.

the antifreeze activity.

Assembly of glyco-peptoid monomer into linear AFGP analogues was accomplished by using standard Fmoc-based solid-peptide synthesis. Briefly, the protected linear glycopeptoids were cleaved from the resin using TFA, and the acetate protecting groups on the carbohydrate were removed by treatment with 1 M NaOH to afford the linear glycopeptoid-AFGP analogues (Table 1). The AFGP analogues 1-6, were assessed for their ability to function as inhibitions of ice recrystallization. The ice recrystallization inhibition (RI) activity of all analogues is presented in Figure 1. All samples were compared to a solution of phosphate buffered saline (PBS, Figure 1(a)) which was used as a negative control for inhibition of recrystallization. The native AFGP8 was included as positive control for ice recrystallization inhibition (AFGP1, Figure 1(b)). Our results show that AFGP3 and 4 which contains the glycopeptoid monomer at second and third position from *N*-terminal respectively were completely lost RI activity. It means that insertion of peptoid monomer at these positions dramatically change the bioactive conformation of AFGP, thereby the interactions between water lattice and ice surface are interrupted. Interestingly, the decreased in the activity was modestly compromised by positioning the peptoid monomer at the *N*-terminal (AFGP2) that results in a dramatic increase in RI activity even though its activity is less than parent. Next, we moved the peptoid monomer into fourth position (AFGP5) and assayed for RI activity. Notably, AFGP5 also displayed the RI activity similar to AFGP2. These results implied that the insertion of peptoid monomer at the *N*- or *C*-terminal keep the interaction between water lattice and ice surface thereby displayed the RI activity. However, insertion of peptoid monomer in the middle of AFGP8 sequence destroyed the interaction between water lattice and quasi-liquid layer of ice. Finally, we substituted all the glyco-threonine with glyco peptoid, resulted in AFGP6 and assayed for RI activity. Except AFGP2 & 5, AFGP6 also failed to show any RI activity. This result was not surprising because peptoid substitution might increase the flexibility of AFGP6 that results in the complete loss of RI activity. Overall, this study provided critical insights on how to position appropriate peptoid fragment to both stabilize the active conformations of AFGPs and contribute to the energetics of interacting with ice/water by analyzing RI activity.

In conclusion, we successfully synthesized the several AFGP analogues bearing *N*-linked glyco-peptoid without any constraints at the glyco-threonine position. In addition, glycopeptoid mimics by solid phase peptide synthesis is an excellent tool to develop simpler AFGP mimics along with the improvement in purity, synthesis time, stability, and yield. Our experimental results clearly indicate that the analogues 2 and 3 (containing glyco-peptoid at first and fourth position from *N*-terminal, respectively) act as a modest inhibitors of ice recrystallization, while analogues 3, 4 and 6 possess no RI activity. Further investigation that is aimed at elucidating the NMR structure of AFGP analogues in under way and the result will be reported in due course. The methodologies presented here would be very useful for the generation of diverse AFGP structural scaffolds by peptoid mimics to investigate the biological significance related to them.

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References

1. Yeh, Y.; Feeney, R. E. *Chem. Rev.* **1996**, *96*, 601.
2. (a) Hachisu, M.; Hinou, H.; Yakamichi, M.; Tsuda, S.; Koshida, S.; Nishimura, S. *Chem. Commun.* **2009**, 1641. (b) Tachibana, Y.; Fletcher, G. L.; Fujitani, N.; Tsuda, S.; Monda, K.; Nishimura, S. *Angew. Chem. Int. Ed*. **2004**, *43*, 856.
3. (a) Ahn, M.; Muragan, R. N.; Nan, Y. H.; Cheong, C.; Sohn, H.; Kim, E. H.; Hwang, E.; Ryu, E. K.; Kang, S. H.; Shin, S. Y.; Bang, J. K. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 6148. (b) Ahn, M.; Sohn, H.; Nan, Y. H.; Muragan, R. N.; Cheong, C.; Ryu, E. K.; Kim, E. H.; Kang, S. W.; Kim, E. J.; Shin, S. Y.; Bang, J. K. *Bull. Korean Chem. Soc*. **2011**, *32*, 3327.
4. Liu, S.; Ben, R. N. *Org. Lett.* **2005**, *7*, 2385.
5. Norgren, A. S.; Budke, C.; Majer, Z.; Heggemann, C.; Koop, T.; Sewald, N. *Synthesis* **2009**, 488.